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Nested-PCR for diagnosis of *Hymenolepis nana* in the patients of Wasit Province

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Abstract---The current study was carried out at Wasit Province / Iraq in cooperation with AL-Karama Teaching Hospital. Samples were collected from many places at Wasit Province. It included 100 stool samples from Iraqi patients conducted during the period from October to December, 2021 to investigate the prevalence of *Hymenolepis nana*. Only 23 stool sample were positive for *H.nana*. These samples were collected from the villages and rural areas of Wasit Province, the villages of Sayed-Shati and Al-Battar, the area of al-Anwar, the peasants, the expatriates, and the Dujaili area. The results showed that the percentage of *H.nana* infection among patients were 23 (23%). With regard to parasitic infection, the highest infection rate was recorded for the group (4-15) years old, as for sex, the percentage of males reached about 78.26% and females 21.74% in this study with no significant differences. According to residence, The highest infection was appeared in the rural areas 75% and the lowest was in the urban areas 25% with a significant differences.

Keywords---*H.nana*, feces, human, nested-PCR.

Introduction

Hymenolepis nana generally known as the dwarf tapeworm, is one of the most common tapeworms of humans, in which the parasite can cause hymenolepiasis. This zoonotic tapeworm has a cosmopolitan distribution with socio-economic and medical significance which may occur in many countries, worldwide (1,2). The life cycle may be direct when the embryonated eggs are ingested with contaminated food, water or indirect, when embryonated eggs ingested by arthropods (beetles and fleas) as intermediate hosts. Humans and rodents are infected when they ingest cysticercoied-infected arthropods (1,3). Autoinfection can also occur when gravid proglottids release eggs inside the gut. Eggs hatch in the small intestine, liberating the oncospheres embryo, which then penetrates the lamina propria of

the intestinal villi (4). The life span of adult worms is 4 to 6 weeks, but internal autoinfection allows the infection to persist for years (5). *Hymenolepis nana* adults are very small, white when alive, body flattened, 25-80 mm long. The scolex bears four suckers and rostellum well developed with a circle of about 20-27 y-shaped hooks, proglottids numerous and transversely elongated. Its eggs are usually spherical, 30-54 μm in diameter, with a thin outer membranous shell and a thick internal embryophore containing the hexacanth embryo, on inner membrane are two small poles from which 4-8 filaments arise and spread out between the two membranes (6,1).

Transmission of infection occurs through fecaloral route by ingestion of eggs from contaminated hands, frequently by contamination of food and water, and rarely from ingestion of food contaminated with fleas harboring the cysticercoid larvae (7,8). Diagnosis is based on the demonstration of the characteristic eggs in the faeces by direct smear, the eggs can readily be concentrated by the salt flotation and formalin-ether sedimentation method (9,10) This study aimed to estimate overall prevalence of *Hymenolepis nana* infections in children at Wasit province and to identify factors associated with infection including gender, age, school-related, parent's jobs and district.

Materials and Methods

Materials

Primers

The PCR primers were used in the present study for detection *Hymenolepis nana* based on small subunit ribosomal rRNA gene were designed in this study using NCBI-Genbank (MH337819.1). These primers were provided from Scientific Researcher. Co. Ltd, Iraq according to primers that used from researcher trsnani *et al.*, 2016 (11), as following table:

Primers	Sequence 5'-3'		Product size
<i>Hymenolepis nana</i> (ssrRNA gene)	F	CAGCATGCATTCACCGCAAT	317bp
	R	TCTCTTCAAGGTGTGGCCAC	

Methods

Ethical Consideration

The proposal of the study was approved by the Ethical Committee of Wasit University, the patients were interviewed and the ones who were willing to participate and sign an informed consent were recruited.

PCR

The PCR technique was performed for detection *Hymenolepis nana* based on small subunit ribosomal RNA gene from stool samples. This method was carried out according to following steps:

Stool DNA Extraction

Sample lysis step

1. A 200mg of the stool sample was transferred to Beadbeating tube containing ceramic beads, and then 800 μ l ST1 buffer and vortex briefly then incubate at 70°C for 5 minutes. Vortex at 10 minutes. Then, centrifuged at 8,000 x g for 2 minutes.
2. 500 μ l supernatant was transferred to a new 1.5 ml microcentrifuge tube

DNA Binding step

1. A 800 μ l ST3 Buffer was added into the flow-through then mix immediately by shaking vigorously for 5 seconds. Then placed a GD Column (green ring) in a 2 ml Collection Tube.
2. A 700 μ l sample mixture was transferred to the GD Column. Then, centrifuged at 16,000 x g for 1 minute.
3. The GD Column back in the 2 ml Collection Tube. The, centrifuged at 16,000 x g for 1 minute.
4. The flow-through was discarded, then place GD Column back in the 2 ml Collection Tube.

Wash step

- a. 400 μ l ST3 Buffer was added into the GD Column. Then, centrifuged at 16,000 x g for 30 seconds at room temperature.
- b. The flow-through was discarded, then place the GD Column back in the 2 ml Collection Tube.
- c. 600 μ l Wash Buffer was added to the GD Column. Then, centrifuged at 16,000 x g for 30 seconds.
- d. The flow-through was discarded, then place the GD Column back in the 2 ml Collection Tube.
- e. The empty GD Column collection Tube was centrifuged at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

Elution step

- 1- The dry GD Column was placed into new 1.5 ml microcentrifuge tube. Add 100 μ l Elution Buffer into column matrix.
- 2- The GD Column was let stand for at least 2 minutes. Then, centrifuged for 2 minutes to elut the purified DNA

PCR product analysis

PCR products were visualized by using UV Transilluminator .

Results

The total number of samples were 100 feces samples collected from people who attended AL-Karamah Teaching Hospital in Wasit province. The results appeared that 23 (23%) of feces samples were positive for *H. nana*.

Table 1: Distribution of *H. nana* infection according to gender in Wasit province

Gender	Positive No. (%)	Negative No. (%)	Total No. (%)
Male	18 (18%)	42 (42%)	60 (60%)
Female	5 (5%)	35(35%)	40 (40%)
Total	23 (23%)	77 (77%)	100(100%)

Table 2: Distribution of *H. nana* infection according to geographic area in Wasit province

Geographic area	Positive No.	Negative No.	Total No. (%)
Rural	20 (20%)	55(55%)	75(75%)
Urban	3(3%)	22(22%)	25(25%)
Total	23(23%)	77(77%)	100(100%)

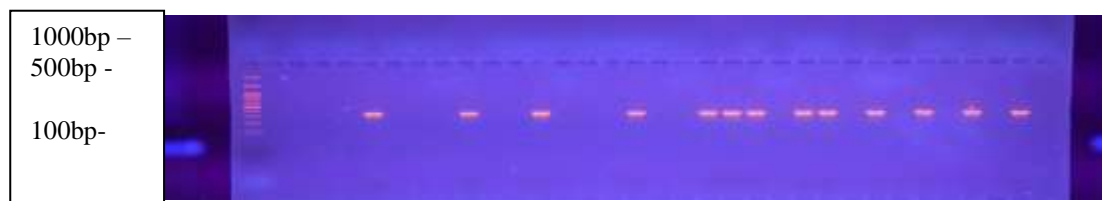


Fig. (1) Gel Electrophoreses by agarose 0.8 % that show the result of PCR of *ssrRNA* gene for diagnosing *H. nana* : Ladder *H. nana* lane was read at 317 bp.

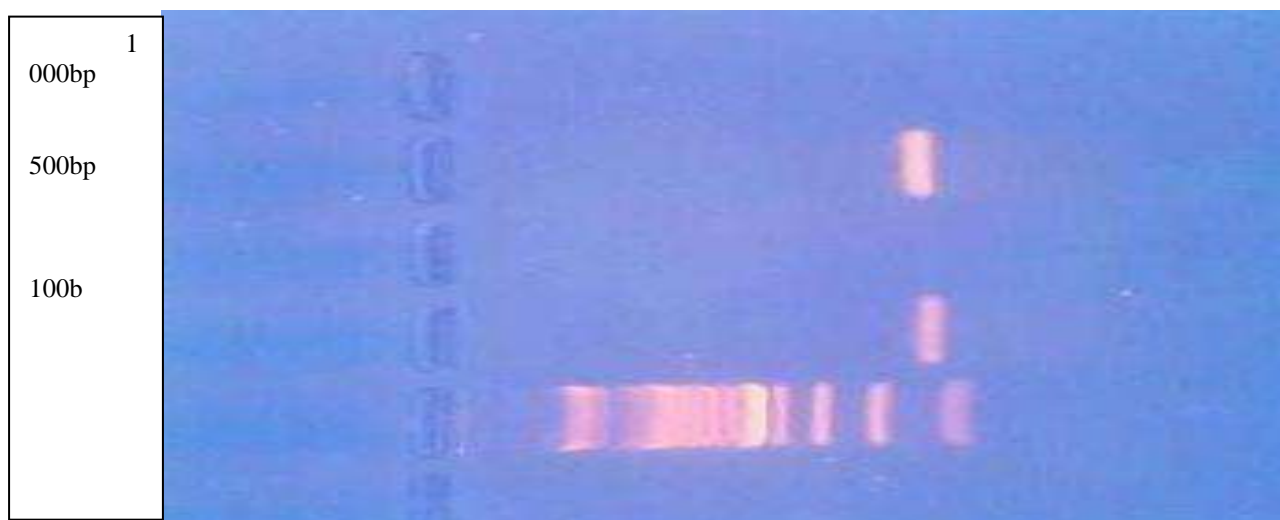


Fig. (2) Gel Electrophoreses by agarose 0.8 % that show the result of PCR of *ssrRNA* gene for diagnosing *H. nana* : Ladder *H. nana* lane was read at 317 bp.



Fig. (3) Gel Electrophoreses by agarose 0.8 % that show the result of PCR of *ssrRNA* gene for diagnosing *H. nana* : Ladder *H. nana* lane was read at 317 bp.

Discussion

Human hymenolepiasis, a disease that occurs throughout the world. Diagnosis and identification of these tapeworms in human patients is usually based on egg morphology in fecal examination (12). However, the identification based on morphology has not been a suitable method in determining the species of tapeworm due to the similarities present in the morphological and phenotypic profile of different stages of the parasite eggs (13). In the current study, we used PCR-sequencing technique for characterization of nuclear rDNA ITS2 gene sequences of *H. nana* to precisely identify the parasite. So far, different studies have been carried out in Iran where *H. nana* infections have been reported. Kheirandish (14),(15) and Badparva *et al.*, (16) in three different studies conducted to determine the prevalence of intestinal parasites in Western Iran, reported *H. nana* infection rates of 0.1%, 0.5%, and 0.3%, respectively(14-16). In a study on 800 schoolchildren in Golestan province, northern Iran, *H. nana* infection rate was 1.5% (17). Similarly, in a study performed in rural region of Orumiyeh, northwest of Iran, the infection rate of the parasite in primary school children was 0.2% (18). In the present study, the highest infection rate was observed in the age group under 30 years old, a finding consistent with that of a previous study on this parasite (19). *Hymenolepis nana* usually causes many clinical symptoms such as headache, weakness, loss of appetite (anorexia), meteorism (bloating, nausea, vomiting, itching, irritability, sleeplessness, enuresis, abdominal pain, and diarrhea) (19). Genetic diversity of *H. nana* has been studied using some genetic makers, such as cytochrome c oxidase subunit 1 (*cox1*) and the first and second internal transcribed spacer (ITS-1 and ITS-2) regions of nuclear ribosomal DNA (rDNA) (20,21). In the current study, all ten positive specimens of *H. nana* successfully multiplied by molecular method and no sequence differences were found in the ITS2 between the isolates.

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